Original Article

A NEW VALIDATED CHIRAL RP-HPLC METHOD FOR THE DETERMINATION OF STABILITY OF MEBEVERINE ENANTIOMERS IN THE PRESENCE OF ITS DEGRADATION PRODUCTS

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ABSTRACT

Objective: An enantioselective reverse phase high performance liquid chromatographic method was developed and validated for the analysis of mebeverine enantiomers. This method was used to investigate the stability and degradation behaviour of mebeverine under different stress conditions recommended by International Conference on Harmonization (ICH).

Methods: An Isocratic chiral RP-HPLC method for the resolution of mebeverine and its degradation products was successfully achieved on a Phenomenex® lux cellulose 1 column, using UV detector at wavelength of 219 nm, with a mobile phase consisting of 0.1% diethyl amine in methanol, 20 mM ammonium bicarbonate (pH: 4.6) adjusted with trifluroacetic acid, 0.1% diethyl amine in isopropyl alcohol (55: 15: 30 v/v/v), and a flow rate of 1.2 ml/min. The drug was subjected to alkaline, acidic, neutral, oxidative, and photolytic conditions in order to mimic stress conditions. The stressed samples were also analysed by this method.

Results: The method developed provided the linear correlation (R^2 =0.999) for the drug between a range of 56–84 µg/ml for (-) mebeverine and 52–78 µg/ml (+) mebeverine respectively. The limit of detection and limit of quantification of (-) mebeverine was found to be 0.30 µg/ml, 0.90 µg/ml and (+) mebeverine was found to be 0.32 µg/ml, 0.97 µg/ml respectively.

Conclusion: The method developed was found to provides good sensitivity and excellent precision and reproducibility and can be applied in the quality control of drug products.

Keywords: Stability-indicating method, Validation, Chiral, Mebeverine.

INTRODUCTION

The stability-indicating method is a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability indicating assay accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities. The presence of impurities and degradation products of drugs can change chemical, pharmacological and toxicological properties of drugs and have significant impact on their quality and safety. Since drugs are, especially, sensitive to environmental factors, strict storage conditions are necessary [1]. The ICH guideline Q1A on stability testing of new drug substances suggests that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and efficacy must be done by validated stability-indicating methods. Stress testing should be carried out on a drug to establish its inherent stability characteristics and to support the suitability of the proposed analytical method. It is also suggested that stress testing should include the effects of temperature, susceptibility across a wide range of pH values, as well as oxidative and photolytic conditions [2].

Similar to stability-indicating studies, enantioselective analytical techniques are important in order to determine the pharmacological and toxicological properties of chiral drugs since there may be significant variability in clinical responses between enantiomers in the same individual. A prerequisite for determining stereo specific pharmacodynamics and pharmacokinetics of optical isomers is the development of an enantioselective analytical methodology. High performance liquid chromatography (HPLC) is one of the well established analytical techniques commonly employed in conducting stability studies and in chiral separation [3]. HPLC has gained popularity due to its high resolution capacity, speed, sensitivity and specificity. Separation of enantiomers by HPLC can be achieved using either chiral stationary phases or chiral additives to the mobile phase. The most common HPLC approach for resolving enantiomers involves the use of chiral stationary phases (CSPs) [4].

Mebeverine hydrochloride is chemically known as 3, 4-dimethoxybenzoic acid 4-[ethyl-2-(4-methoxyphenyl)-1-methyl ethyl] aminobutylveratrate hydrochloride (fig.1) has a direct, nonspecific antispasmodic action on smooth muscle. It is used in the treatment of abdominal pain and spasm associated with gastrointestinal disorders such as mucous colitis [5]. Mebeverine has been used clinically for the treatment of irritable bowel syndrome for many years. A nonaqueous titration method is described in the British Pharmacopoeia (BP) (2008) and this procedure is used for determination of mebeverine in bulk drug. Additionally spectrophotometric determination of mebeverine in tablets is described in BP 2008. [6]. Earlier publications have described a HPLC methodology useful for the estimation of enantiomers in the presence of degradation products formed during forced degradation studies [7].
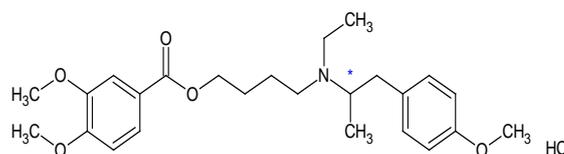

Fig. 1: Chemical structure of Mebeverine hydrochloride

The aim of this present study was to establish a validated stability-indicating chiral HPLC method for mebeverine enantiomers by using phenomenex® lux cellulose 1 column. None of the analytical methods published to date has described stability-indicating method for the reverse phase chiral HPLC method of mebeverine enantiomers in presence of its degradation products.

MATERIALS AND METHODS

Solvents and chemicals

Mebeverine hydrochloride working standard was procured from swapnroop drugs and pharmaceuticals, Aurangabad, Gujarat, India. Methanol, acetonitrile and isopropyl alcohol (HPLC grade),

ammonium bicarbonate were supplied by Merck®, diethyl amine and trifluroacetic acid from S. D. Fine Chemicals. Commercially available mebeverine tablets were purchased commercially from the local market, Udhagamandalam, Tamilnadu, India.

Instrumentation

Chromatographic separation and quantitative determination were performed by using a high performance liquid chromatographic system, from Shimadzu (Kyoto, Japan) equipped with LC-10 AT-VP solvent data station delivery system, an SPD M10 A UV detector, LC-2010 an HT auto sampler with loop volume of 100 µl and the class VP data station was used. A phenomenex® lux cellulose 1 (250x 4.6 mm i. d, 5 µm) column was used for the enantiomeric separation of mebeverine. The mobile phase consisted of 0.1% diethyl amine in methanol, 20 mM ammonium bicarbonate (pH 4.6) adjusted with trifluroacetic acid and 0.1% diethyl amine in isopropyl alcohol (55: 15: 30 v/v/v). The total study was performed at a flow rate of 1.2 ml/min with detection wavelength at 219 nm.

Selection of wavelength

Around 10 µg/ml solutions of mebeverine hydrochloride in methanol were prepared and UV spectrum recorded by scanning the solution in the range of 200 nm to 400 nm. Wavelength of 219 nm was selected from UV spectrum (fig. 2) since this was the maximum absorbance showed for mebeverine hydrochloride.

Preparation of Stock and Standard solution

The standard stock solution of mebeverine hydrochloride was prepared by dissolving 10 mg of the drug in 10 ml volumetric flask with methanol to obtain a concentration of 1 mg/ml and stored in air tight container. Furthermore, the working standard solutions were prepared by diluting with mobile phase to obtain a concentration of (100 µg/ml) respectively. The standard solution was prepared and injected and the chromatogram was recorded (fig. 3).
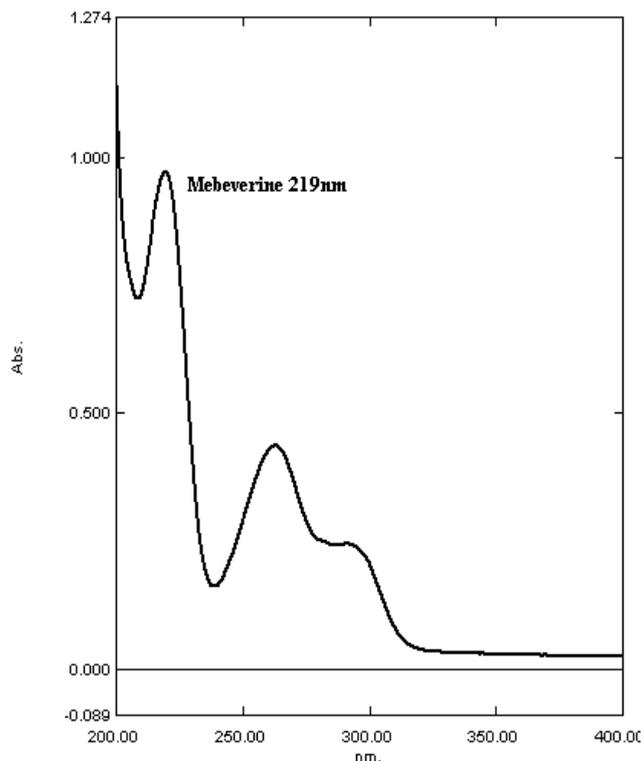

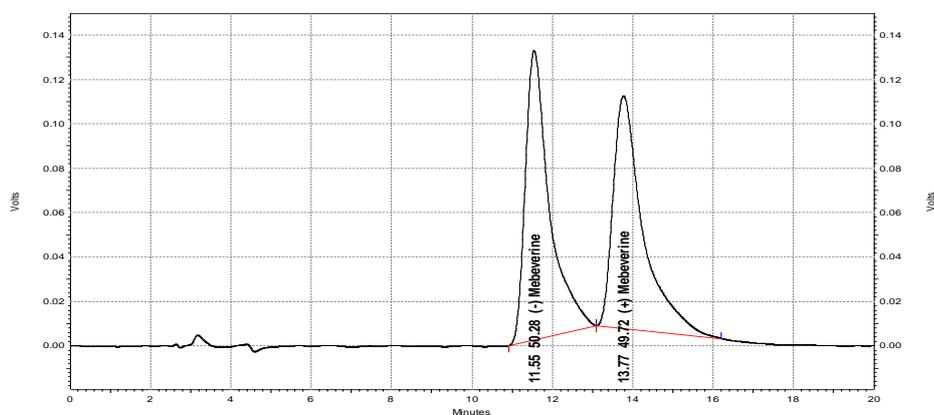**Fig. 2: UV spectrum of Mebeverine hydrochloride (abs = absorbance)**

Fig. 3: Typical HPLC chromatogram of (-) Mebeverine and (+) Mebeverine

Forced degradation studies

Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The forced degradation of the drugs was achieved by the following stress conditions

Base degradation

1 ml of 1000 µg mebeverine hydrochloride sample was taken into 10 ml volumetric flask, and diluted with 0.1N sodium hydroxide solution, contents were mixed well. 1 ml of this solution was taken in 10 ml volumetric flask and neutralized with 1 ml of 1N hydrochloric acid and diluted to 10 ml with mobile phase. These solutions were injected in HPLC system and chromatograms are recorded.

Acid degradation

1 ml of 1000 µg mebeverine hydrochloride sample was taken into 10 ml volumetric flask, and diluted with 1N hydrochloric acid solution, contents were mixed well. 1 ml of this solution was taken in 10 ml volumetric flask and neutralized with 1 ml of 1N sodium hydroxide and diluted to 10 ml with the mobile phase. These solutions were injected in HPLC system and chromatograms are recorded.

Neutral degradation

1 ml of 1000 µg mebeverine hydrochloride samples was taken into 10 ml volumetric flask, and diluted with Milli Q water, contents were mixed well. 1 ml of this solution was taken in 10 ml volumetric flask and diluted to 10 ml with mobile phase. These solutions were injected and the chromatograms are recorded.

Oxidative degradation

1 ml of 1000 µg mebeverine hydrochloride sample was taken into 10 ml volumetric flask, and diluted with 30% hydrogen peroxide, contents were mixed well. 1 ml of this solution was taken in 10 ml volumetric flask and diluted to 10 ml with the mobile phase. These solutions were injected and the chromatograms are recorded.

Photolytic degradation

1 ml of 1000 µg mebeverine hydrochloride sample was taken into 10 ml volumetric flask, and diluted with mobile phase and kept in UV chamber for 24 hrs. 1 ml of this solution was taken in 10 ml volumetric flask and diluted to 10 ml with mobile phase. These solutions were injected and the chromatograms are recorded.

RESULTS AND DISCUSSION

Optimized of chromatographic conditions

The method development was finally optimized with the following conditions: mobile phase consisting of 0.1% diethyl amine in methanol: 20 mM ammonium bicarbonate (pH adjusted to 4.6 using trifluroacetic acid): 0.1% diethyl amine in isopropyl alcohol in the ratio of (55:15:30 v/v/v) and phenomenex® lux cellulose 1 (250x

4.6 mm i. d, 5 µ) column as stationary phase. The analysis was carried out in an isocratic elution mode using a flow rate of 1.2 ml/min, injection volume of 10 µl at room temperature, and detection of analyte was recorded at 219 nm. The chromatogram was recorded using Class VP data station.

Results of forced degradation studies

The HPLC studies of samples obtained on stress testing of mebeverine enantiomers under different conditions suggested the following degradation behaviours (table 1). In basic degradation studies, it was observed that the mebeverine enantiomers were found to show 24.03% and 28.89% of degradation at room temperature with 0.1N NaoH (fig.4). In acidic degradation, it was observed that the mebeverine enantiomers was found to be stable up to 12 hrs, only around 16.82% and 14.58% of the analyte was degraded through 24 hrs and the main analyte was eluted at 11 and 13 min. Whereas in neutral condition around 18.66% and 21.38% decomposition was observed compared to standard drug (fig. 5). In oxidation stress condition around 13.47% and 16.47% were degraded through 24 hrs and photo-degradation 6.89 % and 9.03 % were degraded through 24 hrs respectively. The drug was degraded more in alkaline condition due to the chemical reaction with methoxyl group.

Table 1: Results of Stress degradation studies of (-) Mebeverine and (+) Mebeverine

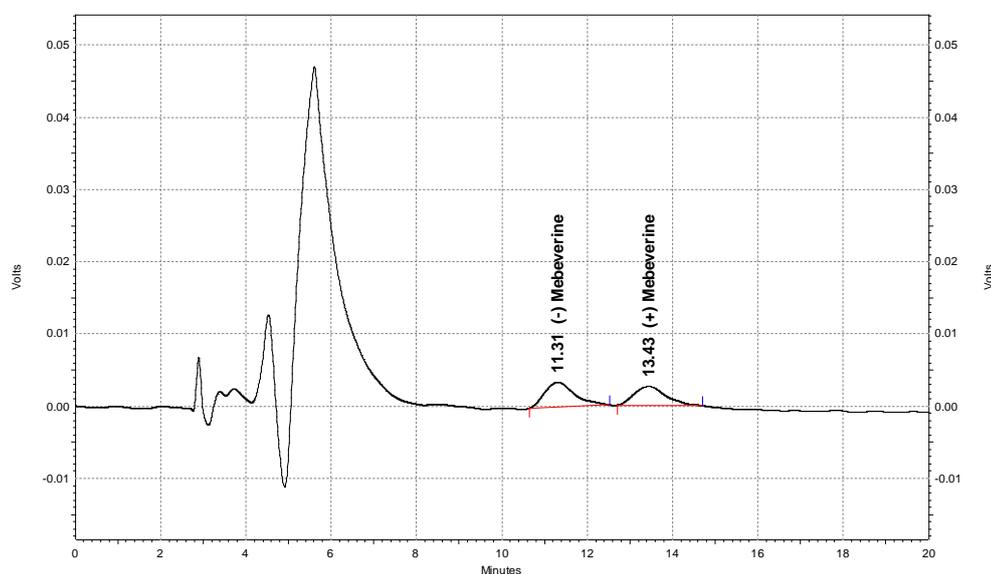| S. No.	Time (hrs)	Basic hydrolysis % degradation 0.1N NaoH		Acid hydrolysis % degradation 1N HCl		Neutral degradation % degradation H_2O		Oxidative degradation % degradation 30% H_2O_2		Photo degradation % degradation UV	
		(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
1	0	0	0	0	0	0	0	0	0	0	0
2	2	5.81	6.85	10.78	5.58	1.88	3.40	3.01	9.47	1.50	0.78
3	4	6.02	8.40	14.46	8.06	15.22	16.19	4.16	12.05	1.61	3.12
4	6	13.04	14.48	15.21	9.61	15.88	20.44	11.10	12.97	4.36	5.53
5	8	15.41	18.99	15.67	11.01	17.41	21.13	11.24	13.33	4.74	6.15
6	12	21.19	24.74	16.05	11.88	18.04	21.21	12.71	14.32	6.18	8.16
7	24	24.03	28.89	16.82	14.58	18.66	21.38	13.47	16.47	6.89	9.03

Fig. 4: Typical HPLC chromatogram of basic degradation samples of Mebeverine enantiomers with 0.1N NaoH at 0 hrs

Method validation

The RP HPLC method was validated according to ICH guidelines. The calibration curves of (-) mebeverine and (+) mebeverine were linear in the range of 56–84 µg/ml and 52–78 µg/ml respectively. The regression equation and correlation coefficient are shown in (fig. 6 (a), (b)). The precision of the method was demonstrated by reproducibility studies. The mean, standard deviation and % RSD

were calculated. The % RSD values were found to be less than 2% revealed that, the method was precise.

The accuracy of the optimized method was determined by absolute recovery experiments. An analysis of the results showed that the percentage recovery values were close to 100% thus establishing that the developed method is accurate and reliable. Detection limit and quantification limit of (-) mebeverine and (+) mebeverine were

found to be 0.30 µg/ml, 0.90 µg/ml and 0.32µg/ml, 0.97µg/ml respectively. No marked changes in the chromatograms occurred on changing the instrument. Chromatographic conditions indicated that the developed method was rugged and robust. The column

efficiency, resolution and the peak asymmetry were calculated for the standard solutions. Signal to noise ratio of the 3 and 10 are generally considered as the limit of detection (LOD) and limit of quantification (LOQ) respectively (table 2).
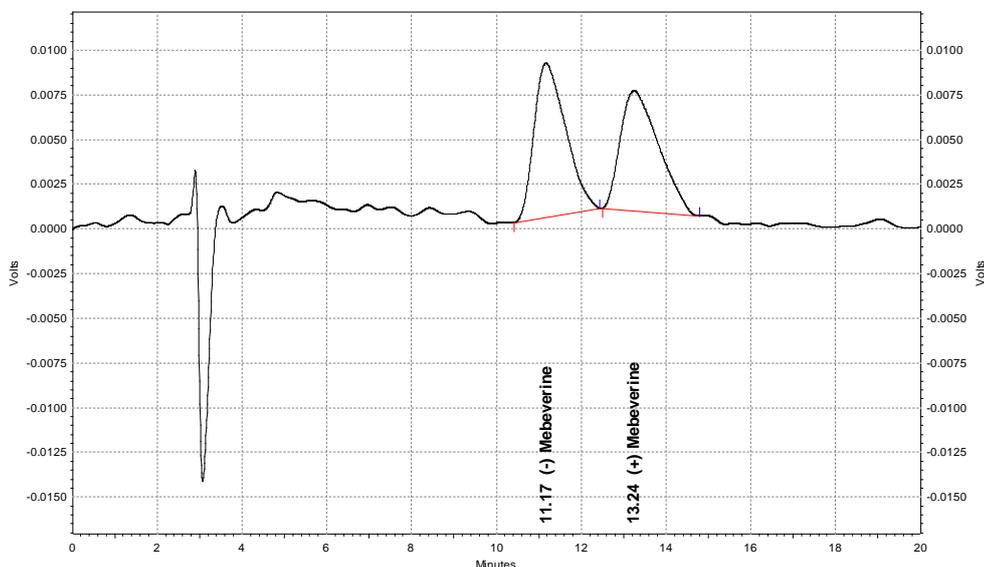

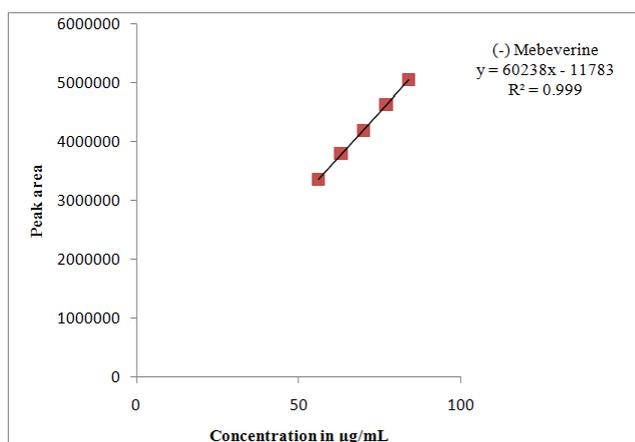**Fig. 5: Typical HPLC chromatogram of neutral condition of Mebeverine enantiomers at 24 hrs.**

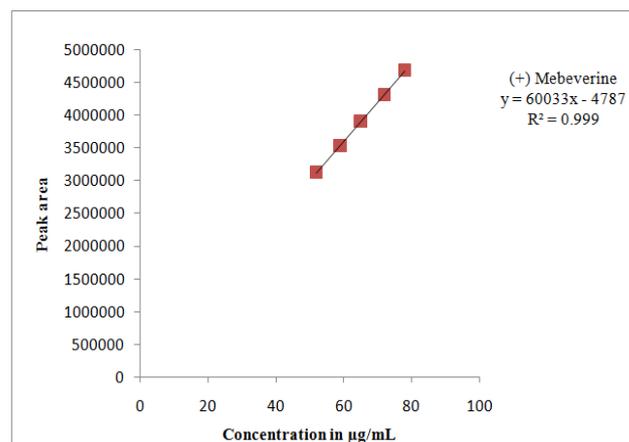

Fig. 6(a): Calibration curve of (-) Mebeverine

Fig. 6(b): Calibration curve of (+) Mebeverine

Table 2: System suitability studies for the determination of Mebeverine enantiomers

S. No.	Parameters	(-) mebeverine	(+) mebeverine
1	Linearity range	56-84 µg/ml	52-78 µg/ml
2	Regression equation y=mx+c	Y= 60238x-11783	Y= 60033x-4787
3	Correlation coefficient	0.999	0.999
4	Theoretical plate/meter	4258	4678
5	Resolution factor	1.8	
6	Asymmetric factor	1.25	1.45
7	LOD (µg/ml)	0.30	0.32
8	LOQ (µg/ml)	0.90	0.97

CONCLUSION

A highly specific stability–indicating chiral HPLC method was developed for the quantification of mebeverine enantiomers in presence of their degradation products. The enantioseparation was carried out by the use of cellulose based chiral column. The total run time for the developed method is 20 min. The method

provides good sensitivity and excellent precision and reproducibility.

blessings and the facilities provided to complete the research work successfully.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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